BABA GHULAM SHAH BADSHAH UNIVERSITY

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(SYNOPSIS FOR REGISTRATION FOR DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY)

Proposed Topic : Conservation biology of three overexploited medicinal plants of North -West Himalayan region.

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BACKGROUND AND JUSTIFICATION:

Plants sustain human life, therefore, their conservation and sustainable utilization is essential. Conservation regimes are being pursued all over the world, including India, with the objective of conserving important medicinal and food plants for posterity. The emphasis is more on plants in danger of getting extinct for various reasons, natural or anthropogenic. These factors may be anthropogenic, demographic or natural. Medicinal plants are second only to food plants in sustaining mankind. Cultivated as they are over millions of hectares of land all over the world, food plants are in no danger of getting extinct (Zohary and Hopf, 1988). Domestication has ensured their survival though in forms very different from their wild allies. On the contrary medicinal plants, save a few that have been cultivated, are exploited from nature. The demand for green medicine and its preference over synthetic drugs has accentuated the demand for medicinal plants. Total dependence on wild populations for ruthless exploitation has caused severe reduction in the size of natural populations. The seriously affected have come to the brink of extinction. Many have become rare. Populations of many species are falling below minimum viable size (Lewis, 1988, Nantel *et al*., 1996; Sheldon *et al.,* 1996). The principle of sustainable utilization is not required as much for any other group of plants as it is for medicinal plants. For conservation of threatened plants, including medicinal plants, two strategies are followed at national as well as at global level:

i) ***In situ*** **conservation**: Attempts of conservation of plant species in their natural habitat. It is accomplished by establishment of protected areas such as National parks, sanctuaries, biosphere reserves, nature reserves etc.

ii) ***Ex situ*** **conservation:** In *ex situ* conservation, germplasm of the plant species to be conserved is maintained at sites away from their natural habitat. *In vivo* and *in-vitro* methods are used for *ex –situ* conservation. *In-vivo* methods attempt raising plants from seeds and vegetative propagules collected from nature under ex-situ conditions, in

botanic gardens, herbal gardens etc. *In- vitro* methods refer to storage of germplasm as seeds, vegetative propagules, pollen etc. at low temperature under aseptic conditions.

Species specific conservation strategy can be drawn only after ascertaining biological and ecological background of the species being tried for conservation. Most medicinal plants have restricted distribution, and are highly habitat specific, found only in forests and occupying highly specialized ecological niches. There is neither biological information nor adequate knowledge of causal factors underlying their rarity in nature (Pushapangadan, 1992). An important aspect of conservation is to know the past and present distribution of the species, its reproductive capacity and the source of threat to its survival in nature (Chandel *et al*., 1996). Other aspects of conservation programmes are habitat preservation and conservation of genetic diversity of rare and threatened species (Frankel and Soule, 1981; Simberloff, 1988). Loss of variation reduces the ability of populations to adapt to changing environments and increases their susceptibility to pest and disease pressures (Fisher, 1930; Hamilton, 1982; Beardmore, 1983).

Species proposed to be studied: Three medicinal plants of himalayan region namely *Valeriana wallichii* DC.(Valerianaceae), *Picrorhiza kurrooa* Royle ex Benth.(Scrophulariaceae) and *Ferula jaeschkeana* Vatke.(Apiaceae) are proposed to be used in the proposed investigation. Reports of their being overexploited from nature, ambiguity about their exact status, and some interesting aspects of their population structure and reproductive biology have come to light during the preliminary studies undertaken in our laboratory which has prompted the proposed study.

***Valeriana wallichii* DC. (Valerianaceae)**

The species is indigenous to India. Commonly known as Indian valerian or Tagar, the plant is an inhabitant of the temperate zone of North- Western Himalaya. It grows upto an altitude of 3,000m (Gilani *et al*., 2005). The extract of its rhizome is a rich source of valepotriates, which have sedative and tranquilizing properties. The plant has for long been used in Indian, unani and allopathic systems of medicine as sedative, antispasmodic, stomachic and stimulant. It is also known to cure disorders of the nervous system (Mathur and Ahuja, 1990). The entire demand of domestic and International drug industry is met from the wild populations which have, over the years, diminished in size

as well as number (Mathur and Ahuja, 1990). The plant grows at moist shady slopes that are easily accessible to man and livestock and are prone to landslides.

ii) *Picrorhiza kurrooa* Royle ex Benth. (Scrophulariaceae)

It is an endangered herb of great medicinal value found in alpine Himalaya between 2,800- 4,800m (Chandra *et al.*, 2006). Rhizome of the plant yields hepatoprotective picrosides (picrosides – 1, II and III) and kutkosides, which are used in ayurvedic preparations and in modern system of medicine as stomachic, purgative, brain tonic and in treatment of liver disorders (Chandra *et al*., 2006). On account of incessant collection its availability in nature has diminished. The species figures in the endangered list drawn by IUCN (Chandra *et al*., 2006). Endangered status, existing importance and growing demand of the species call for appropriate measures for conservation, protection and rehabilitation of the species. Studies undertaken for conservation of the species revolve mostly round *in vitro* propagation through tissue culture (Lal *et al*., 1988; Chandra *et al*., 2004) and raising plants from seed or stolons (Chandra *et al*., 2006). Like *Valeriana*, in *Picrorhiza* too, such aspects as ecology and reproductive potential of plant in nature has been neglected so far. Moreover there is still ambiguity regarding its exact status. Although it has been included in IUCN Red data list as endangered (Nayar and Sastry, 1990), some later reports suggest its exclusion from the category (Kala, 2004).The species grows in open meadows and slopes which are subjected to grazing and are prone to landslides.

iii) *Ferula jaeschkeana* Vatke. (Apiaceae)

Ferula jaeschkeana is a tall herb widely distributed in Jhelum valley and Drass region of J&K state. The plant thrives in the subfreezing temperatures of Drass where it is used by the locals as firewood and medicine. The resin of this plant has wound healing properties and is used in folk medicines for treatment of tumors, chronic wounds and ulcers in man as well as in animals(Khalilova and Saidkhodzhaev, 1998). Seeds are reported to contain essential oil. The plant calls for conservation due to its exploitation and restricted distribution.

Objectives of the study:

To,

a) assess the magnitude and nature of threat to the three species referred above.

b) Investigate their reproductive biology, population structure, genetic system and ecology.

c) Evolve strategy for their conservation.

i) *ex situ,* in the Lead Botanic Garden of the University, and

ii) *in vitro* under aseptic conditions through tissue culture, if required.

Methodology

1) For assessment of the cause and magnitude of threat to the species in nature, field work shall be undertaken to determine number of individuals in a population (population size), density, area of occupancy and possible factors of threat to the species under reference and comparing these values to those laid by IUCN for classifying the plants into categories raised as per the threat they face.

2) Data will be collected on floral morphology, phenology, pollen viability, pollen – ovule ratio, pollination mechanism, pollinator variability and their comparative efficiency, pollen- pistil interaction, and reproductive output using following tools and methodology:

I. **Floral morphology**: studies on the structure and distribution of flowers on the plant in field and laboratory.

II. **Floral -biology**: Data on anthesis will be collected, under *in-situ* as well as *ex-situ* conditions. Anther-dehiscence and stigma receptivity will be periodically checked from fresh and fixed flowers of varying age after staining them with mixture of acid fuchsin, light green and lactic acid (after Lewis, 1979) and by Aniline blue fluorescence method (Shivanna and Rangaswamy, 1993).

III. **Pollen- Ovule ratio**: Pollen output per flower will be calculated by first counting the number of pollen grains per anther and multiplying this figure by the number of stamens per flower. The number of pollen divided by number of ovules per flower will yield pollen-ovule ratio for the species.

IV. **Pollen-stainability and viability**: These will be determined by subjecting freshly released pollen to FCR reaction TTC and acetocarmine staining test. In vitro pollen germination experiments will also be attempted.

V. **Pollination-Mechanism**: Mode of pollination will be ascertained through visual observation, bagging-experiments and hanging slide technique. Hanging slide technique involves suspending glass slides smeared with Mayer's albumen from T-shaped wooden stands all around the plants at varying distances. Presence of significant number of pollen grains of the species under study on the hanging slides confirms wind pollination. In case of entomophilly, insects visiting the flowers will be pursued for their foraging behaviour. They will be collected, identified and classified as visitors, robbers or pollinators on the basis of their activity and pollen load on their body parts. The plants will be checked for the nature of attractants and rewards offered by their flower. Their influence on pollinator activity will be determined by field studies and experimentation.

VI. **Seed set:** will be estimated from plants growing in nature and ex-situ. Percentage seed set will be calculated as follows:

$$\textbf{Percentage seed set} = \frac{\textbf{Number of seeds / plant}}{\textbf{Number of ovules / plant}} \times 100$$

VII. **Seedling survival:** will be estimated by germinating seeds in sterilized soil, on moist filter paper and by sowing them in experimental beds in Botanic garden.

3) Population Structure: population structure of all the three species will be studied by following the methods used by Kalliovirta *et al*., 2006

4) Genetic system: will be worked out by studying the meiotic and breeding systems of plants of each species.

5) Evaluating the Genetic diversity : Genetic diversity will be analysed by following the protocol elaborated below:

DNA – extraction: Leaf samples will be collected from plants of populations located at different geographical sites in the himalayan region, wrapping them in aluminum foils and storing them in liquid nitrogen till final use. CTAB method proposed by Doyle and Doyle in 1987 with modifications wherever necessary shall be followed for genomic DNA isolation.

Marker analysis: After purification and quantification**,** the extracted DNA shall be subjected to marker analysis. The marker systems proposed to be used include:

a) Random amplified polymorphic DNA (RAPD) and

b) Inter simple sequence repeats (ISSR).

Both these marker systems are PCR based. Therefore, properly purified and quantified DNA samples will be subjected to amplification using random primers under standardized reaction conditions. The data will be obtained by scoring gels and the results will be subjected to appropriate statistical tests to determine the extent of genetic variability at inter as well as intra population levels in all three species(Yeh *et al*., 1997; Schneider *et al.,* 2000; Felsentein, 2000; Mantel, 1967 and Cavalcanti, 2001). This will help us in identifying the sites hosting maximum genetic variation of the three species and in devising the strategies for conservation of these sites and species.

Expected Outcome: Once completed the investigation will,

i) establish the magnitude of threat to the three species and help in referring them to appropriate category of threatened plants,

ii) unfold the mechanism of reproduction of the three species and bring to light bottlenecks, if any, in their reproduction.

iii) unravel the extent of genetic diversity in natural populations and help in identifying sites hosting maximum variability for use in conservation of such sites and the species.

iv) Help in designing appropriate conservation strategy for all these taxa.

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